Studies on the Serum Haptoglobin Level in Hemoglobinemia and Its Influence on Renal Excretion of Hemoglobin

By Carl-Bertil Laurell and Margareta Nyman

Jayle found that on addition of a small amount of hemoglobin, serum shows weak peroxidase activity with an optimum around pH 4.4 in spite of the fact that no such activity can be demonstrated in either substance by itself. In later investigations in association with Polonovski he demonstrated that a complex between hemoglobin (Hb) and a serum protein is responsible for this peroxidase activity. This protein, which was not isolated until recently, was called haptoglobin (Hp) because of its interaction with hemoglobin (Hb). In a series of investigations Polonovski, Jayle and associates (for references see Jayle and Boussier) determined the variation of the plasma haptoglobin in health and in some diseases. The haptoglobin concentration was assessed with the aid of a titrimetric method permitting determination of the amount of serum necessary for optimal peroxidase activity of a given amount of Hb. In contrast to the ordinary peroxidases, which act in the presence of H2O2, determination of the complex, HpHb, requires the presence of alkyl peroxides. The concentration of the haptoglobin is expressed by the so-called haptoglobin index, which is a function of the amount of Hp present in a given volume of serum. In a series of French papers and a Dutch monograph it was shown that the Hp index can be used as an indicator of the degree of activity of various diseases. In all conditions characterized by increased tissue disintegration, such as acute and chronic infections, malignant processes or necrosis, the Hp index was found to increase with the activity of the disease. This was also found to be so in rheumatic affections and nephrosis. Subnormal haptoglobin values, on the other hand, have received only little attention, but have been observed in cases of hemolytic conditions and acute hepatitis. In many large French hospitals determination of the Hp index has been used as a routine clinical method for the same purpose as the erythrocyte sedimentation rate in other parts of Europe and in U. S. A.

Jayle and Boussier succeeded in preparing haptoglobin (II) from urine from a child with nephrosis. This protein proved to belong to the group of α2 mucoproteins. Seventeen per cent of the molecule consisted of carbohydrate expressed as galactose, mannose and N-acetyl-glucosamine. The sedimentation constant was found to be 4.1 Svedberg units and the molecular weight about 85,000. This protein contained no measurable amount of phosphorus, cholesterol, lipids or glucuronic acid. Other analytic data including the solubility of the protein are...
SERUM HAPTOGLOBIN LEVEL IN HEMOGLOBINEMIA

given in the publications referred to above. It has thus been shown, for example, that it is not precipitated by 0.6 N perchloric acid but by 0.5 N trichloracetic acid, which indicates that this haptoglobin should be assigned to the group of mucoproteins determinable with Winzler's technic. As yet the quantities of Hp isolated from serum have not been sufficient for closer study, but the complex hemoglobin-haptoglobin has been isolated from serum and analyzed with ultracentrifugal and diffusion methods. The molecular weight computed from experimental data was found to be 310,000, as against 155,000 expected. The ratio between the iron:protein-content and the molecular weight suggested that the complex contained two molecules of hemoglobin and one molecule of a haptoglobin of twice the molecular weight of the purified haptoglobin (II) isolated from urine. Jayle et al. therefore expressed the view that the haptoglobin molecule first isolated from urine is a rare form (II) and that the haptoglobin (I) normally occurring in serum is probably a dimer of Hp (II).

According to Jayle et al., it is mainly the absolute increase in the haptoglobin that is usually responsible for the increase in the \( \alpha_2 \) globulin. An exception to the rule is nephrosis, in which other \( \alpha_2 \) components are also markedly increased.

Experimental and clinical observations suggest that Hp is a depolymerization product of the ground substance of the connective tissue and that an increased plasma Hp suggests either increased connective tissue formation or increased connective tissue disintegration in the organism.

Wieme and Tuttle found that the addition of Hb to serum before electrophoresis would change the distribution of the proteins in the \( \alpha-\beta \)-region. According to Wieme, on addition of Hb the normal \( \alpha_2 \) component decreased and a new component appeared between \( \alpha_2 \) and \( \beta_1 \). In the presence of a high concentration of Hb two separate Hb-containing fractions were obtained, one between \( \alpha_2 \) and \( \beta_1 \), and one in \( \beta_1 \). At pH 8.9 free Hb has the same electrophoretic mobility as the \( \beta_1 \) components. Wieme stated that the intermediary fraction consists of the complex HpHb. He considered it important that the degree of hemolysis should be taken into account in the evaluation of the \( \alpha_2 \) values of serum containing Hb.

Smithies observed that hemoglobin can form one, two or three complexes with \( \alpha_2 \) components in one and the same normal serum. These complexes differ in electrophoretic mobility in starch gel. He also found that sera from healthy persons can be divided into 3 hereditary groups according to the number and mobility of the complexes found with hemoglobin.

These results suggest that haptoglobin embraces a group of serum mucoproteins characterized by their power to bind hemoglobin.

ELECTROPHORETIC METHOD FOR DETERMINATION OF HAPTOGLOBIN

Principle of Performance

We have devised an electrophoretic method for quantitative determination of the haptoglobin concentration in serum. In order to secure good separation between the HpHb complex and the free hemoglobin, the experiments were carried out in a phosphate buffer at pH 7. Under these experimental conditions
HbIIb migrates towards the anode and free Hb towards the cathode. Hb would show hardly any mobility at this pH unless the endosmotic waterflow in the paper used carried the fraction towards the cathode.

Hemoglobin was added in increasing quantity to a series of tubes containing a standard amount of serum. All of the samples were then examined by paper electrophoresis. After the strips of paper had been dried they were colored with leucomalachite green dissolved in dilute acetic acid in the presence of hydrogen peroxide. Those parts of the paper containing hemoglobin turned green.

The largest quantity of Hb bound in the anode fraction indicates the concentration of the haptoglobin fraction in the serum expressed as the hemoglobin-binding capacity of the α-globulins.

**Results and Discussion**

Figure 1 gives a diagram of the distribution and intensity of the color obtained on analysis of a serum whose haptoglobin can bind 100 mg. hemoglobin 100 ml. In the serum mixture “110” more hemoglobin (10 mg./100 ml.) was added to the serum than could be taken up by the Hp. If hemoglobin is added to serum in a concentration far in excess of the hemoglobin-binding capacity of the haptoglobin, the anode fraction will not increase but only the cathode fraction, which shows that the haptoglobin has a high affinity for Hb at physiologic pH and, further, that the Hp fraction is saturated as soon as any free Hb becomes demonstrable. A complete report on the methodologic details will be published elsewhere.

The breadth of the leucomalachite colorable anode fraction (fig. 1) decreases and the color intensity increases with increasing amounts of hemoglobin added to the serum until the haptoglobin is no longer able to bind further amounts of hemoglobin. After addition of hemoglobin in a total amount not exceeding the hemoglobin binding capacity of the serum leucomalachite colorable fraction is broader than that of a pure protein after electrophoresis under the standard conditions used. Not until the hemoglobin binding protein is saturated with Hb does the breadth of the colored fraction correspond to that of a single homogenous component. This phenomenon argues strongly against the Hp molecule.
being able to bind only one molecule of Hb. If, however, Hp could bind more than one molecule of Hb, various complexes with varying mobility would appear on addition of Hb below the saturation value. If Hp contained two active radicals the distribution of the components on 50 per cent saturation would be Hp (1) HpHb (2) HpHb₂ (3). Since every molecule of HpHb₂ has two peroxidase active groups, the peroxidase activity of one molecule of HpHb₂ should reasonably be twice as large as that of one molecule HpHb, i.e., the same intensity of color should be obtained in the region corresponding to the mobility of HpHb as in that corresponding to HpHb₂ in the example given. It can thus be deduced that every molecule of the normally, quantitatively dominant haptoglobin can bind at least two molecules of Hb.

Electrophoresis combined with peroxidase coloration can also be used to demonstrate small quantities of methemalbumin in serum. Replacement of the phosphate buffer by barbiturate buffer (pH 8.6) increases the sensitivity of the method. On electrophoretic separation of Hb, HpHb, HpHb₂, and Hp in barbiturate buffer at pH 8.6, the Hb migrates in a, Hp in α₂ and the complex in the intermediate zone. The separation of Hb, HpHb₂ is, however, less sharp, for which reason it is difficult to decide exactly at which hemoglobin concentration free Hb first appears.

The Normal Haptoglobin Level of Serum

An investigation of the variation in haptoglobin in different diseases is in progress, but already now we can report that haptoglobin measured as the quantity of hemoglobin that can be bound in normal plasma varies between 0.14 and 0.05 Gm. per 100 ml. (Mean value 0.09, n = 30). Assuming the molecular weight of HpHb₂ given by Jayle to be 310.000, these limits correspond to 0.18 and 0.065 Gm. Hp per 100 ml. serum (Mean value: 0.115). Jayle, who converted his index values into absolute figures, gave the normal concentration of Hp as 1.3 ± 0.5 per cent of the total proteins in the serum, i.e., about 0.1 ± 0.04 Gm. per 100 ml. serum, which shows that the peroxidatic and the electrophoretic method give values of the same order for normal sera.

The Haptoglobin Level after Intravenous Injection of Hemoglobin

Hitherto the biologic relationship between haptoglobin and hemoglobin has received less attention. Workers in the field have used mainly the affinity of Hp for Hb as an aid for determining the concentration of Hp and evidently not investigated whether Hp can serve as an Hb carrier in vivo. This might possibly be explained by the fact that earlier methods available for determination of haptoglobin could only be used to demonstrate the interaction between Hb and Hp in weak acid solutions. The degree of affinity between Hb and Hp could not be investigated in the physiologic pH-range by these methods. The electrophoretic experiments showed, however, that haptoglobin binds hemoglobin in a firm complex also at a pH of 6-8.6. It may therefore be assumed that it will also do so in vivo, i.e., on intravascular hemolysis and on intravenous administration Hp will take up Hb. Analysis of sera from some patients with acquired hemolytic icterus and pernicious anemia (untreated) showed no demonstrable Hp. This
suggested a possible relationship between hemoglobin and haptoglobin metabolism. In an attempt to examine this possibility we studied the concentration of the hemoglobin, the hemoglobin binding capacity, the electrophoretic protein changes and other variables after i.v. administration of Hb. As yet, only three such series of experiments have been carried out, but as the results obtained showed complete agreement, they were considered to justify publication.

Methods

Hemoglobin was determined according to Crosby and Furth. The haptoglobin concentration was determined and expressed as the Hb binding capacity of serum according to the principles given above. Hemoglobin (10 mg/100 ml. at a time) was added in increasing quantity to a series of aliquots of each serum. The methodologic error was about ±5 mg. per 100 ml.

Paper electrophoresis was done according to Laurell, Laurell and Skoogi. The $\alpha_2$-globulin value was obtained by cutting the colored electrophoretic strip in between the $\alpha_1$ and $\alpha_2$ fractions and immediately in front of the $\beta$ fraction (fig. 5). All samples belonging to the same series were stained and washed together.

Serum bilirubin was determined according to Jendrassik & Gröf.

Preparation of Hemoglobin for Intravenous Use

Red cells were repeatedly washed on the centrifuge with physiologic saline. The red cell sediment was frozen at $-15^\circ$ C. and then allowed to thaw, after which 2 volumes of distilled water was added. The suspension was centrifuged at high speed to get rid of red cell ghosts. The solution was made isotonic by addition of saline and sterilized by filtration.

Hemoglobin Infusion Experiments

Case L. The initial hemoglobin binding capacity of the serum was found to be 85 mg. per 100 ml. Plasma volume computed from body weight was 3.43 l and per body height 3.48 l. The approximate plasma volume was thus 3.45 l. This corresponds to a hemoglobin binding capacity of 2.93 Gm. hemoglobin. 2.93 Gm. Hb (9.2 per cent solution) was slowly (10 minutes) injected intravenously. The times at which blood samples were collected and the results of the analysis are apparent from the tables and figures. The injection produced no symptoms or side effects whatsoever. The benzidine reaction of the urine remained negative after the injection, but a qualitative urobilinogen test was slightly positive.

1.5 Gm. Hb had been injected into the same individual one month earlier, when the hemoglobin binding capacity was found to be 55 mg. per 100 ml. The results were in principle identical.

Case N. The initial hemoglobin binding capacity of the serum was found to be 115 mg. per 100 ml. Plasma volume computed from the body weight was 3.1 l and per height 2.7 l. Approximate plasma volume 2.9 l. This corresponds to a hemoglobin binding capacity of 3.35 Gm. hemoglobin. 3.35 Gm. Hb (9.6 per cent solution) was slowly (10 minutes) injected intravenously. The times at which blood samples were collected and the results of the analysis are apparent from the tables and figures. The injection produced no symptoms or side effects whatsoever. The benzidine reaction of the urine remained negative after the injection, but a qualitative urobilinogen test was slightly positive.

Results and Discussion

The results are given in the figures and the tables. From figures 2 and 3 and table 1 and 2 it is apparent that the quantity of Hb injected was found in the serum bound to the haptoglobin in the serum sample collected 10 minutes after
the injection. As expected, in Case 1 (L.) the haptoglobin was saturated with Hb. Case N. showed a somewhat smaller increase in plasma Hb than expected, but this can be explained if it be assumed that since N. had a slightly subnormal red cell count, the theoretic plasma volume was probably fictitiously low.
Table 1.—Case L. Serum Analysis after Intravenous Injection of Hemoglobin

<table>
<thead>
<tr>
<th>Time in relation to Hb i.v.</th>
<th>Electrophoretic analysis</th>
<th>Serum Hb (mg./100 ml.)</th>
<th>Serum bilirubin (mg./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free Hb</td>
<td>Hb-binding capacity (mg./100 ml.)</td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td></td>
<td>85</td>
<td>1.5</td>
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<tr>
<td>Minutes after</td>
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<tr>
<td>10</td>
<td>-</td>
<td>++ +</td>
<td>86</td>
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<td>190</td>
<td>-</td>
<td>++ (+)</td>
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<tr>
<td>370</td>
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<td>(+)</td>
<td>13</td>
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<td>3</td>
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<td>6</td>
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Table 2.—Case N. Serum Analysis after Intravenous Injection of Hemoglobin

<table>
<thead>
<tr>
<th>Time in relation to Hb i.v.</th>
<th>Electrophoretic analysis</th>
<th>Serum Hb (mg./100 ml.)</th>
<th>Serum bilirubin (mg./100 ml.)</th>
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</thead>
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<tr>
<td></td>
<td>Free Hb</td>
<td>Hb-binding capacity (mg./100 ml.)</td>
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</tr>
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<td>Minutes after</td>
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<td></td>
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<td>++ (+)</td>
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<td>(+)</td>
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<td>Days after</td>
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<td>-</td>
<td>80</td>
<td>5</td>
</tr>
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</table>

Figure 4 shows the rate at which the hemoglobin in plasma decreased. It is apparent that the hemoglobin from the plasma of both individuals was eliminated at a constant rate until low values were reached.

Figures 2 and 3 show that the Hb and the Hb binding capacity decreased at the same rate. The latter gradually recovered during the following week.

The protein concentration in the electrophoretic α2-region increased after injection and then decreased successively during the Hb-elimination phase (figs. 5 and 6). After the main part of the hemoglobin had been eliminated the α2 level was significantly lower than before the injection. The electrophoretic α2-fraction was cut out with such a breadth (fig. 7) as to comprise the conven-
tional \( \alpha_2 \) and the intermediate fractions between \( \alpha_2 \) and \( \beta_1 \) containing the complexes HbHPb and HbHb\(_2\).

It may be added that none of the samples contained free Hb in measurable quantities and that no hemoglobin was found in the urine. The serum bilirubin

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**Fig. 4**—Plasma hemoglobin values noted at various intervals after intravenous administration of Hb. Upper curve, case X, lower, Case L.

**Fig. 5**—\( \alpha_2 \)-globulin concentration before and after intravenous injection of hemoglobin. Case L.
level was slightly increased (table 1), probably due to the increased hemoglobin catabolism. During the daytime the bilirubin normally decreases following the intake of food as in the present cases.

These results suggest some conclusions of metabolic interest and are discussed below.

Free Hb does not occur in plasma after i.v. injection until more Hb has been administered than the quantity that can be bound by the circulating haptoglobin.

The entire HpHb₂-complex is eliminated from the blood stream. Theoretically, the heme component might be split off from the HpHb₂-complex and eliminated from the blood stream and leave behind, for example, a peroxidatically inactive Hp-globin complex. However, the changes observed in the α₂ concentration during the elimination phase (figs. 5 and 6) militated against such an assumption. For comparison with the observed values, 3 theoretic values of the α₂-

![Graph showing α₂-globulin concentration before and after intravenous injection of hemoglobin. Case N.](image)

**Fig. 6.**—α₂-globulin concentration before and after intravenous injection of hemoglobin.

![Electrophoretic protein pattern (pH 8.6) before and after injection of hemoglobin.](image)

**Fig. 7.**—Electrophoretic protein pattern (pH 8.6) before and after injection of hemoglobin.
concentration have also been plotted in these figures. If the Hb is taken up by the haptoglobin, the \( \alpha_2 \) value should logically increase in proportion to the amount of Hb injected. It can be shown with the aid of the molecular weights for Hb and HpHb\(_2\) that during the elimination phase the \( \alpha_2 \) value will reach its original level when about 44 per cent of the Hb has been eliminated, if the total HpHb\(_2\) complex is eliminated. The \( \alpha_2 \) value at the time when all the Hb has been cleared can also be computed if it be assumed that no regeneration of Hp has occurred in the meantime. The observed values are in good agreement with the theoretical despite the errors of the method.

The changes in the appearance of the electrophoretic protein distribution within the \( \alpha_2 \)-region (fig. 7) after the injection of Hb was in accord with those observed by Wieme.\(^4\) The main \( \alpha_2 \)-band decreased, but a new band was found between \( \alpha_2 \) and \( \beta_1 \). This band then faded successively during the elimination phase and the bulk of \( \alpha_2 \) remained unchanged, which also supports the assumption that the entire HpHb\(_2\) complex leaves the circulation to be catabolized somewhere or other.

It is apparent from figure 4 that the hemoglobin concentration in plasma decreases at a constant rate until low values are reached. In both individuals studied the serum Hb-values decreased at a rate of 13 mg. per 100 ml. per hour. The constant elimination rate during the main part of the elimination period suggests that some organic system (probably R.E.S.) that takes up HpHb\(_2\) has a low capacity, because otherwise the elimination curve should reasonably have been exponential. The elimination constant, which can readily be determined from a few plottings, may serve as an index of the functional capacity of this system.

Our observations suggest that the minimum amount of Hb i.v. necessary to produce hemoglobinuria will vary with the amount of circulating haptoglobin. The Hp-values recorded during the week following the Hb-injection suggest that the rate of Hp production is of the same magnitude as that for most globulins.

**Renal Excretion of Extracorporeal Hemoglobin**

The biologic effect of Hb administered intravenously has been the subject of investigations for several decades. As early as 1916 Sellard and Minot\(^9\) showed that the injection of hemoglobin from 20 ml. hemolysed red cells could be tolerated well without any systemic side effect. Later investigators\(^11, 12\) increased the doses considerably. Although these doses were followed by massive hemoglobinuria they seldom produced any alarming systemic side effects. The effect of hemoglobin on renal function has recently been studied with modern clearance tests by Miller and McDonald.\(^13\) They found, however, that a small dose (0.5 Gm.) Hb produced a vasopressor effect with renal vasoconstriction and simultaneous increase in the percentage of glomerular filtrate reabsorbed by the renal tubules. These results were confirmed by Demaria and Harris,\(^15\) who showed that administration of Mg\(^++\) counteracted the influence of the hemoglobin on the circulation and on water reabsorption in the kidneys. Miller et al.\(^12\) also found that the glomerular permeability to hemoglobin averaged 13 per cent of that to inulin. According to these authors, the term “renal threshold” is a value.
dependent upon three variables, viz. glomerular permeability, glomerular filtration rate and tubular recovery. As a rule hemoglobinuria was not observed in healthy persons until the serum concentration exceeded a level of about 100–135 mg. per 100 ml.\textsuperscript{16} This was ascribed to a relatively high tubular recovery of hemoglobin. This capacity was given by McDonald et al.\textsuperscript{14} as 17.1 mg. per 100 ml. of glomerular filtrate. However, as far back as 1914 Sellard and Minot\textsuperscript{19} showed that the minimum amount of hemoglobin i.v. necessary to produce hemoglobinuria varied widely and was smaller in pernicious or hemolytic anemia than in health. Lichty, Havill and Whipple\textsuperscript{17} found repeated administration of Hb intravenously to result in a lowering of the renal threshold. Ottenberg and Fox\textsuperscript{18} found that the degree of hemoglobinuria in normals did not vary in proportion to the plasma hemoglobin-concentration.

Our experiments showed that Hb added to plasma is strongly bound by Hp. The molecular weight of this complex (HpHb\textsubscript{2}) is about 310,000. However, since such large molecules can pass out in the primary urine only extremely slowly, Hb molecules will hardly pass out into the primary urine until more hemoglobin has been liberated or administered intravascularly than can be bound by Hp. As mentioned, the normal Hb binding capacity of haptoglobin lies with a wide spread around about 90 mg./100 ml., i.e., just below the level which Ottenberg and Fox\textsuperscript{18} and others have claimed to represent the threshold level of the kidneys. Hence, generally speaking, all experimental determinations of the renal Hb clearance on record are fictitiously low because it has been presupposed that all hemoglobin in plasma occurs as free hemoglobin. This implies that the tubular reabsorbing capacity has been overestimated.

The level of Hb in the primary urine must be related to the level of free Hb in plasma. Thus, assuming that the haptoglobin in one person can bind 300 mg. Hb/100 ml. and in another none, administration or intravascular release of 310 mg. Hb/100 ml. in the latter case would not produce a greater increase in free Hb than would 10 mg./100 ml. in the former. In view of the re-absorbing capacity of the tubules, hemoglobinuria should reasonably occur in hemoglobinemia only when hemoglobin is liberated or administered intravascularly in excess of what can be bound by the haptoglobin or when the amount of free Hb occurring in the primary urine exceeds the amount that can be re-absorbed. This “threshold” value of plasma Hb, which must be reached before hemoglobinuria occurs, must be ascribable in part to the Hp-content of the serum and in part to the reabsorption capacity of the kidneys. Judging by the level of the “renal threshold” values on record in normals, it is the Hp-concentration that is the dominant of these two determinants. The change in the renal threshold observed first by Lichty et al.\textsuperscript{17} after repeated Hb-administration can be ascribed to the decrease in Hp concentration obtained after administration of Hb, because our experiments showed that Hp is eliminated together with Hb and, secondly, that Hp is regenerated relatively slowly. Then intravascular liberation of hemoglobin or intravenous administration of hemoglobin should thus result in the occurrence of Hb in the primary urine already at low hemoglobin concentration in the serum. The fundamental experimental work on the mechanism of hemoglobinuria was carried out by Lichty, Havill, Whipple and associates in 1932.\textsuperscript{17} They introduced the term minimal renal threshold as the expression of the largest
amount of hemoglobin which could be tolerated on daily administration of Hb without the occurrence of hemoglobinuria. They found that if hemoglobin was injected intravenously to an untreated dog and the dose was such that the final concentration in the blood was less than that corresponding to the minimal renal threshold, no hemoglobin or iron would be histologically demonstrable in the tubules. This observation can be explained if it be assumed that the amount of hemoglobin injected in these experiments was equal to or less than the amount that could be bound by haptoglobin. If this doses was somewhat increased, hemoglobinuria did not occur, but then Hb and Fe were demonstrable in the tubules. This suggests that in these experiments the amount of Hb injected was so large that the concentration in the blood exceeded the binding capacity of haptoglobin but not of the reabsorption-capacity of the tubules. Further data are given by Whipple and associates in their informative studies, which can now be regarded as supporting the assumption that haptoglobin in plasma is one of the variables that determines the amount of Hb that can be tolerated intravenously without the occurrence of hemoglobinuria.

Analysis of the literature showed that as far back as in 1916 Sellard and Minot presented data suggesting the importance of haptoglobin. They found that the production of hemoglobinuria in patients with pernicious and hemolytic anemia did not require such a large dose of Hb as did normals. “We only wish to point out that there is a definite relationship between the tolerance of hemoglobin and the blood destruction.” This most interesting observation has later been forgotten but is probably true since serum from patients with hemolytic and pernicious anemias have no hemoglobin binding capacity.

**SUMMARY**

A short survey is given of the literature on haptoglobin, the hemoglobin-binding serum protein, its properties and biologic variations. The principles of an electrophoretic method for quantitative determination of the serum haptoglobin are described.

Electrophoretic studies showed that haptoglobin has a high affinity for hemoglobin at physiologic pH and that every haptoglobin molecule can bind at least 2 hemoglobin molecules.

Observations made following the intravenous injection of hemoglobin showed:

- that hemoglobin administered intravenously is bound by the haptoglobin;
- that free hemoglobin is not demonstrable until more hemoglobin has been injected than can be bound by the haptoglobin;
- that the complex hemoglobin-haptoglobin is eliminated from the plasma after intravascular hemolysis or intravenous administration of hemoglobin without being excreted in the urine;
- that the hemoglobin-haptoglobin complex is removed from the plasma at a constant rate during the major part of the elimination period;
- that the haptoglobin level will fall to nil within 24 hours, if the amount of hemoglobin injected is sufficient to bind all the haptoglobin available. During the following days the rate of formation of haptoglobin can be studied.

From the data available it can be concluded that hemoglobinuria cannot
appear until the amount of hemoglobin administered intravenously or the amount liberated intravascularly exceeds the binding power of the haptoglobin and the reabsorption capacity of the tubules. The variation observed by earlier authors in the so-called renal threshold for hemoglobin on intravenous injection of hemoglobin can be explained among other things by the variation in the haptoglobin content in one and the same subject, i.e., if the haptoglobin level is low, the threshold value will also be low, and vice versa.

**Summario in Interlingua**

Es presentate un breve revista del litteratura super haptoglobina, le proteina seral que liga le hemoglobina, super su proprietates e su variationes biologic. Es describite le principios de un metodo electrophoretic pro le determination quantitative del haptoglobina seral.

Studios electrophoretic ha monstrate que haptoglobina ha un alte affinitate pro hemoglobina a pH physiologic e que omne molecula de haptoglobina pote ligar al minus 2 molecuras de hemoglobina.

Observationes facite post le injection intravenose de hemoglobina monstrava (1) que hemoglobina administrate per injectiones intravenose es ligate per le haptoglobina; (2) que libere hemoglobina non es demonstrabile usque un quantitate de hemoglobina es injicite que es plus grande que le quantitate que pote esser ligate per le haptoglobina; (3) que le complexo hemoglobina-haptoglobina es eliminate ab le plasma post hemolyse intravascular o post le administration intravenose de hemoglobina, sin esser excretate in le urina; (4) que le complexo hemoglobina-haptoglobin es eliminate ab le plasma a un rapiditate constante durante le major parte del periodo de elimination; e (5) que le nivello de haptoglobina descende a zero intra 24 horas, si le quantitate de hemoglobina injicite es sufficiente pro ligar omne le disponibile haptoglobina. Durante le sequente dies le rapiditate del formation de haptoglobina pote esser studiate.

Ab le datos disponibile on pote concluder que hemoglobinuria non pote apparer usque le quantitate de hemoglobina administrate intravenosemente (o le quantitate liberate intravascularmente) excede le capacitate ligative del haptoglobina e le capacitate reabsorptive del tubulos. Le variationes observate per previe autores in le si-appellate limine renal pro hemoglobina con le injection intravenose de hemoglobina pote esser explicate per—inter altere causas—variationes in le contento de haptoglobina in le mesme subjecto, i.e.: quando le nivello de haptoglobina es basse, le valor del limine renal es etiam basse, e vice versa.

**REFERENCES**

3b ——: Personal communication.


Studies on the Serum Haptoglobin Level in Hemoglobinemia and Its Influence on Renal Excretion of Hemoglobin

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